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THE INFLUENCE OF STREPTOCOCCUS ALLER-  
GEN AND STREPTOLYSIN O ON THE FORMA-  
TION OF ANTITETANUS IMMUNITY

L. A. Pozhidaeva-Sinitsina

Foreign Technology Division  
Wright-Patterson Air Force Base, Ohio

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By: L. A. Pozhidayeva-Sinitsina

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Г г	Г г	G, g	У у	У у	U, u
Д д	Д д	D, d	Ф ф	Ф ф	F, f
Е е	Е е	Ye, ye; E, e*	Х х	Х х	Kh, kh
Ж ж	Ж ж	Zh, zh	Ц ц	Ц ц	Ts, ts
З з	З з	Z, z	Ч ч	Ч ч	Ch, ch
И и	И и	I, i	Ш ш	Ш ш	Sh, sh
Я я	Я я	Y, y	Щ щ	Щ щ	Shch, shch
К к	К к	K, k	Ь ь	Ь ь	"
Л л	Л л	L, l	Н н	Н н	Y, y
М м	М м	M, m	҃ ҃	҃ ҃	"
Н н	Н н	N, n	҃ ҃	҃ ҃	E, e
О о	О о	O, o	҃ ҃	҃ ҃	Yu, yu
П п	П п	P, p	҃ ҃	҃ ҃	Ya, ya

\* ye initially, after vowels, and after ъ, ѣ; e elsewhere.  
 When written as є in Russian, transliterate as yѣ or є.  
 The use of diacritical marks is preferred, but such marks  
 may be omitted when expediency dictates.

THE INFLUENCE OF STREPTOCOCCUS ALLERGEN AND  
STREPTOLYSIN O ON THE FORMATION OF ANTITETANUS  
IMMUNITY

L. A. Pozhidayeva-Sinitsina

Odessa Medical Institute

The recent works of Soviet and foreign investigators confirm the important role of the products of the vital activity of streptococcus in the development of the infectious process and immunity.

Considering that the properties of the streptococcus allergen and streptolysin O significantly influence the reactivity of the organism, and also the fact that the development and course of tetanus, complicated by added streptococcus infection, takes on a different nature, it is of interest to study the influence of streptococcus allergen and streptolysin O on the development of immunity following immunization with tetanus toxoid.

The investigations were made on 60 guinea pigs in the weight group of 300-400 g; of these 15 animals served as a control group. All the control and test guinea pigs received single subcutaneous doses of 37 ES [EC - possible meaning - standard unit] of native tetanus toxoid in a volume of 0.5 ml. In the experiment we studied the influence of streptococcus allergen and streptolysin O, and also the combined effect of these two preparations on the immune

antitetanus reorganization of the body. For this purpose the test animals were divided into three groups of 15 each.

One day prior to immunization with tetanus toxoid the pigs in the first group each received 10 ml of streptococcus allergen subcutaneously. The same amount of allergen was administered on the day of immunization, and on the 3rd 5 ml more. The pigs in the second group in the indicated periods received subcutaneously 150 doses each of streptolysin O. The animals of the third group received half doses of streptococcus allergen and streptolysin O in the same periods. The control group of guinea pigs received only tetanus toxoid.

The check of the state of immunity in the animals of the control and test groups was made by titration of antitoxin in the serum and based on the survival rate of guinea pigs, when after termination of the period of immunization they received 50 DLM of tetanus toxin.

The animals were observed for two months.

During the period of immunization the guinea pigs of the control and test groups were weighed. Here it turned out that the streptococcus allergen and streptolysin O, and also the combination of these two preparations cause a lowering of weight in the test animals during the first week of the test.

The subsequent stage of the work was the determination of the content of antitoxin in the serum of the animals of all the groups. The blood for the obtaining of serum was taken from the heart of the animals at the end of the third week of immunization.

Determination of the titer of antitoxin began with the establishment of the test dose of working series of dry tetanus toxin. To 0.1 AU [AE - antigen/antitoxic unit] of standard antitetanus serum in a volume of 0.2 ml decreasing doses of toxin were added.

Table. The influence of streptococcus allergen and streptolysin O on the formation of antitetanus immunity.

Group of animals	Change of weight during the experiment ( $M \pm m$ )	Titers of antitoxin in serum in AU	Course of tetanus intoxication		
			healthy	I degree	II degree
First (received the allergen)	$-41,0 \pm 8,66$ $P < 0,01$	$+46,6 \pm 14,2$ $P < 0,05$	9 $P < 0,05$	2 $P < 0,05$	— $P < 0,05$
Second (received streptolysin O)	$-44,4 \pm 3,84$ $P < 0,01$	$+52,8 \pm 9,75$ $P < 0,025$	12 $P < 0,025$	1 $P < 0,025$	— $P < 0,025$
Third (received allergen and streptolysin O)	$-52,2 \pm 4,58$ $P < 0,01$	$+35,44 \pm 12,6$ $P < 0,025$	9 $P < 0,025$	— $P < 0,025$	— $P < 0,025$
Control	$+14,0 \pm 2,93$	$+93,6 \pm 6,88$	6 7	6 2	— 1

in a volume of 0.2 ml. The mixture was held for 45 min at a temperature of 37°, after which 0.4 ml in volume was administered to white mice which weighed 16-18 g. For each dose of toxin 4 mice were used. They were observed for 4 days.

As the test dose we took the same minimal amount of toxin, which in a mixture with 0.1 AU of standard antitetanus serum in 4 days after subcutaneous administration caused the death of more than 50% of the mice.

For determination of the titer of the test serum a test dose of standard toxin, taken in a volume of 0.2 ml, was added to whole serum also in a volume of 0.2 ml. The mixture of toxin and serum was set for neutralization in a thermostat at a temperature of 37° for 45 min, after which 0.4 ml doses were administered subcutaneously to white mice. The titer of the serum was calculated, proceeding from its dilution, which in the case of administration with the test dose of toxin protected 100% of the mice from death for four days.

All told in the test 46 sera were checked. The determination of titer of antitoxin in whole serum was done in connection with the insufficient amount of it. In the serum of 9 guinea pigs out of the 11 which received injections of streptococcus allergen the titer of antitoxin was lower than 0.5 AU, and in the sera of the remaining two it was equal to 0.5 AU.

In the sera of the 12 guinea pigs of the second group, which received streptolysin O, the titer of antitoxin was below 0.5 AU, and only in the serum of one guinea pig was it equal to 0.5 AU.

In the sera of all 9 animals of the third group, which received the streptococcus allergen together with streptolysin O, the titer of antitoxin was below 0.5 AU.

In the control group in the sera of 7 pigs the titer of antitoxin was equal to 0.5 AU, and in the sera of the 6 remaining it was below 0.5 AU (see table).

During statistical processing the difference in the titers of the animals of the control and test groups was statistically reliable ( $P < 0.05$ ,  $P < 0.025$ , and  $P < 0.025$  respectively).

Thus streptococcus allergen and streptolysin O, and also a combination of them, exert an inhibiting influence on the development of antitoxin in the body of animals which had been immunized with tetanus toxoid.

For clearing up the level of development of immunity the pigs of the test and control groups received 500 DLM each of tetanus toxin one month after their immunization with tetanus toxoid. Observations of the animals were conducted for 6 days with a calculation of the developing clinical aspects of tetanus intoxication.

The results of the tests showed that the pigs of the control group acquired a more persistent immunity. Thus out of 13 pigs 6 remained healthy, 2 pigs were in a state of tetanus intoxication degree II, 1 - degree III, and 4 animals died.

Tetanus intoxication in the test animals proceeded more severely and basically terminated in a lethal outcome. Thus out of 11 guinea pigs which received streptococcus allergen and were immunized with tetanus toxoid, 8 died with the presence of the typical symptoms of tetanus intoxication, and 3 were found in a state of tetanus intoxication degree I. Of the 13 pigs which received streptolysin O 9 died, the remainder were found in a state of tetanus intoxication of degrees I, II, and III. Of the 9 animals which received streptococcus allergen and streptolysin O, 8 died and one pig was found in a state of tetanus intoxication degree I (see table).

The difference in the clinical course of tetanus intoxication of animals in the test and control groups is statistically reliable ( $P < 0.05$ ,  $P < 0.025$ , and  $P < 0.05$  respectively).

Thus the inhibiting influence of streptococcus allergen and streptolysin O on the formation of immunity to the toxin of tetanus is confirmed by the more severe clinical course of tetanus intoxication and the greater mortality among test animals in comparison with control.